Attorney Docket No. 15907-0022 PATENTS

CERTIFICATE OF MAILING

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June 28, 2002

#17
8-1-02

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

O'Brien et al. :

App. No.: 09/728,716 : Art Unit: 1615

Filed: November 30, 2000 : Examiner: Gollamudi S. Kishore

For: Radiation Sensitive Liposomes

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

DECLARATION PURSUANT TO 37 CFR §1.132

I, David F. O'Brien, Ph.D., hereby declare as follows:

1. I am a Professor of Chemistry at the University of Arizona and co-inventor of the invention disclosed and claimed in the above-referenced patent application, filed November 30, 2000. I consider myself to be skilled in the art of organic and polymer chemistry. My *curriculum vitae* is appended hereto (Exhibit A).

2. I am familiar with the specification and claims in the above referenced patent application. I have reviewed the amended claims which require the polymerizable colipids to be in the form of discrete domains.

3. I am a co-author of the article by Lamparski *et al.* titled "Photoinduced Destabilization of Liposomes", Biochemistry, vol. 31(3), 685-694 (1992) and I am thoroughly familiar with the subject matter of the teachings of Lamparski.

4. Lamparski discloses the stability of two-component liposomes composed of polymerizable 1,2-bis-[10-(2',4'-hexadienoyloxy)decanoyl]-*sn*-glycero-3-

phosphatidylcholine ("SorbPC") and nonpolymerizable lipid that is either a dioleoylphosphatidylethanolamine ("DOPE") or dioleolyphosphatidylcholine ("DOPC") via fluorescence leakage assay. Lamparski teaches the utilization of the photopolymerization of lipid bilayers where the exposure to the ultraviolet light causes SorbPC-containing liposomes to form poly-SorbPC and results in phase separation to yield a polymerized and nonpolymerized bilayers.

5. Lamparski *only* describes the light-induced destabilization and aqueous contents leakage of liposomes composed of DOPE and SorbPC. It further discloses that photoinduced polymerization does *not* destabilize the liposomes composed of DOPC and SorbPC (see Figure 2 and Table 2).

6. In Lamparski, liposomes composed of DOPE or DOPC in either a 2:1 or 3:1 molar ratio with bis-SorbPC were prepared by extrusion, reverse-phase evaporation and mild sonication of hydrated bilayers.

7. The fluorescence leakage assays were performed on liposomes with established calcein permeability assay. These experiments were performed at 25 °C. The experiments measured the loss of monomeric SorbPC during photopolymerization and the percent leakage of calcein. The percent leakage of calcein was calculated from the increase in calcein fluorescence intensity.

8. The experimental data in Lamparski showed ultraviolet irradiation of the liposomes composed of DOPE and bis-SorbPC increased the membrane permeability. However, ultraviolet irradiation of the liposomes composed of DOPC and bis-SorbPC did not increase the membrane permeability even after greater than 80% polymerization (see page 689).

9. Hydrated bilayers of a pure lipid exist in a solid-like state when the experimental temperature is below the main phase transition temperature ("T_m") of the lipid. Mixtures of lipids are also in a solid-like state when the sample temperature is near or below the T_m of the lowest melting lipid in the mixture. Mixtures of lipids in the solid-like phase separate into discrete domains enriched in the individual lipids, whereas at higher temperatures the lipids are more liquid-like and the discrete domains are less likely to form.

10. The main phase transition temperature of DOPC is –20 °C. The main phase transition temperature of DOPE is –10 °C. The main phase transition temperature of the bis-SorbPC used in the Lamparski study is 27 °C. Therefore, the liposomes prepared and studied in Lamparski were designed to form fluid phase liposomes at room temperature and above. Furthermore, at 3:1 molar ratio with bis-SorbPC, the global transition temperature would have been substantially below the room temperature. These conditions favor random

mixing of the lipids and bis-SorbPC where polymerizable colipids are randomly distributed throughout the liposomal membrane.

11. The T_m values for hydrated bilayers of the individual purified lipid are obtained by differential scanning calorimetry ("DSC"). Table 1 shown below lists T_m values for dipalmitoylPC ("DPPC"), distearoylPC ("DSPC"), and diarachidoylPC ("DAPC"), the PC's bearing two 16, 18 and 20 carbon acyl chains, respectively. The Table also show the T_m values for two polymerizable bis-SorbPC lipids and pegylated distearoylPE ("PEG-DSPE"). The subscripts indicate the number of atoms in length of the acyl chains that bear the sorbyl group.

Table 1

Hydrated Lipids	Main Phase Transition Temperature (T_m)
DPPC	41.5 °C
DSPC	54.5 °C
DAPC	66 °C
Bis-SorbPC$_{17,17}$	29 °C
Bis-SorbPC$_{19,19}$	42.5 °C
PEG-DSPE	60 °C
PEG-DOPE	0 to 10 °C

12. Formation of discrete domains is one of the methods that can be used to achieve a non-random distribution of the lipids. Discrete domains may be formed by lowering the sample temperature below the T_m of the individual lipids. Alternatively, the liposomes can be prepared from lipids that each have a T_m above the experimental temperature. Consequently, the lipids are in a solid-like phase during the experiment and are less likely to mix with one another. For example, when bis-SorbPC$_{17,17}$ is combined with DSPC, discrete domains are formed at room temperature.

13. In the present invention, the T_m temperatures of various hydrated lipids, as shown in Table 1 above, were used to design room temperature solid-like liposomes composed of bis-SorbPC$_{17,17}$ and either DPPC or DSPC, plus the PEG-DSPE. The specific examples in the specification of the subject application (pages 36-38) illustrates the effects of ionizing

radiation on polymerizable colipids. Various liposomes were prepared including those composed of lipids, such as DSPC, and colipids, such as bis-SorbPC$_{17,17}$. As stated above, when bis-SorbPC$_{17,17}$ is combined with DSPC, discrete domains are formed at room temperature.

14. For liposomes prepared from Composition 1 (page 36 of the specification) comprising lipids in particular DOPC and bis-SorbPC$_{17,17}$, significant release of liposomal contents was observed at ionizing radiation doses of 250 rads. The global transition temperature of this liposome would have been substantially below the room temperature. Therefore, this condition would favor random mixing of the lipids and bis-SorbPC where polymerizable colipids are randomly distributed throughout the liposomal membrane.

15. For liposomes prepared from Composition 2 (page 36 of the specification) comprising lipids in particular DSPC and bis-SorbPC$_{17,17}$, significant release of liposomal contents was observed at ionizing radiation doses as low as 50 rads, which is substantially lower compared to 250 rads for Composition 1. The condition of Composition 2 would create discrete domains when the Composition is prepared at room temperature, because of the phase separation.

16. Increasing release for Composition 1 was observed with increasing doses of ionizing radiation up through 2500 rads. In contrast, increasing release for Composition 2 was observed up through 200-250 rads (pages 36-37 of the specification).

17. These results clearly indicate that a liposome delivery system comprising a lipid and a polymerizable colipid in the form of discrete domains within the liposome enhances the release of the liposomal contents with minimal irradiation.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above identified application or any patent issued thereon.

So declared,

David F. O'Brien 6/27/02

David F. O'Brien Date

VITA

David F. O'Brien

Born on November 18, 1936, in Litchfield, Illinois

Married (Nancy); one son, two daughters

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EDUCATION

A.B. in Chemistry 1958:	Wabash College
PhD. in Physical Organic Chemistry 1962:	University of Illinois

PROFESSIONAL EXPERIENCE

1962-1973	Research Laboratories, Eastman Kodak Company Physical Organic Laboratory Spectral Sensitization Laboratory
1973-1974	Sabbatical Assignment University of California at Berkeley
1974-1986	Research Laboratories, Eastman Kodak Company Organic Photochemistry Laboratory
1987-1989	Professor of Chemistry University of Arizona
1989-date	Professor of Chemistry and Biochemistry University of Arizona
1995	Visiting Professor Mar Planck Institut für Polymerforschung

RESEARCH INTERESTS

Preparation and characterization of supramolecular assemblies of lipids and polymerizable lipids; crosslinking stabilization of nonlamellar lipid phases and formation of organic zeolites; novel chemistries based on the capability of bilayer assemblies to sequester and/or laterally organize reactants; polymerization induced templating in supramolecular assemblies: stable discotic mesophases; interaction of peptides and proteins with lipid bilayer membranes.

PROFESSIONAL AFFILATIONS

American Chemical Society
American Association for the Advancement of Science
Biophysical Society
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AWARDS

Fellow of the American Association for the Advancement of Science

STUDENTS AND POSTDOCTORAL ASSOCIATES *(1987 to present)*

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Mr. David Frankel	9/87-5/88	
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Ms. Rebecca Goldman	10/89-5/90	
Mr. Eric Oblinger	2/90-5/92,	
Ms. Linda Nett	6/90-8/90	REU
Ms. Patty Roosa	6/91-8/91	REU
Ms. Crystal Bernstein	6/91-8/91	REU
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Ms. Camille Dalke	10/92-9/93	
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Mr. Jeff Schultz	9/95-5/96	
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Ms. Shannon McLean	9/96-7/98	
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Mr. Doyle Bennett	2/89- 2/95	PhD	Advanced Materials Corp.
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Mr. Britt Minch	1/00-	present	
Mr. Robert Schmid	1/00-	present	
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Dr. Krishnudu Kasireddy	9/99- 11/01	

PUBLICATIONS

Publications Submitted Prior to Appointment at the University of Arizona in 1987

1. D. E. Applequist and D. F. O'Brien. 1963. Equilibria in Halogen-Lithium Interconversions. **J. Am. Chem. Soc.** *85*, 743-748.

2. D. F. O'Brien and J. W. Gates. 1965. The Configurations of the 2,3-Epoxides of Some Diels-Alder Adducts of 1,4-Benzoquinones. **J. Org. Chem.** *30*, 2593.

3. D. F. O'Brien and J. W. Gates. 1966. Some Reactions of 3-Hydroxypyrazole. **J. Org. Chem.** *31*, 1538.

4. M. J. Youngquist, D. F. O'Brien, and J. W. Gates. 1966. Mechanism of the Thermal Isomerization of Some Diels-Alder Adducts of 1,4-Benzoquinone 2,3-Epoxides. **J. Am. Chem. Soc.** *88*, 4960.

5. D. F. O'Brien. 1968. The Kinetics of the Reaction of 1-Phenyl-1H-tetrazole-5-thiol Sodium Salt with Substituted 1,4-Benzoquinone 2,3-Epoxides. **J. Org. Chem.** *33*, 262.

6. D. F. O'Brien. 1973. Spectral Sensitization of Evaporated Silver Bromide by Monomolecular Layers of Cyanine Dyes. **Photogr. Sci. Eng.** *17*, 226.

7. D. F. O'Brien. 1974. J-Aggregation in Monomolecular Layers of Cyanine Dyes. **Photogr. Sci. Eng.** *18*, 16.

8. T. M. Kelly and D. F. O'Brien. 1974. Nanosecond Fluorescence Spectrometry in Photographic Systems. **Photogr. Sci. Eng.** *18*, 68.

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10. D. F. O'Brien and L. F. Costa. 1976. Photochemical Functionality of Rhodopsin-Phospholipid Recombinant Membranes. **Invest. Opthal. Visual Sci.** *17*, 85.

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27. D. F. O'Brien. 1982. Lipid Dependence of Rhodopsin Kinetics. **Methods in Enzymology Vol. 81** *Biomembranes*, ed. L. Packer, Academic Press, N.Y. p.378.

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35. K. Dorn, R. T. Klingbiel, D. P. Specht, P. N. Tyminski, H. Ringdorf, and D. F. O'Brien. 1984. Permeability of Polmerized Bilayer Membranes from Methacryloyl and Butadiene Lipids. **J. Am. Chem. Soc.** *106*, 1627-1633.

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37. P. N. Tyminski, L. H. Latimer, and D. F. O'Brien. 1985. Rhodopsin in Polymerized Bilayer Membranes. **Biophys. J.** *47*, 234a.

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Publications Submitted Since Joining the University of Arizona in 1987.

46. T. Kuo and D. F. O'Brien. 1988. Free Standing Polydiacetylene Films from Bilayer Membranes. **J. Am. Chem. Soc.** *110*, 7571-7572.

47. D. F. O'Brien and U. Liman. 1989. Polymerized Model Biomembranes. **Angewandte Makromolekulare Chemie** *166/167*, 21-38.

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49. T. Kuo and D. F. O'Brien. 1989. Free Standing Polydiacetylene Films. **ACS-Polymer Preprints** *30*, 255-256.

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51. T. Kuo and D. F. O'Brien. 1990. Synthesis and Properties of a Diacetylenic Glutamate Lipid. **Macromolecules** *23*, 3225-3230.

52. D. F. O'Brien. 1990. Reconstitution of Proteins in Polymerized Bilayer Assemblies. **J. Cellular Biochem.** *14B*, 351.

53. T. Kuo and D. F. O'Brien. 1990. Effect of Lipid Structure on the Formation of Ordered Cast Films of Polydiacetylenes. **J. Chem. Soc., Chem. Comm.**, 839-841.

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55. T. Kuo and D. F. O'Brien. 1991. Cast Polydiacetylenic Films from Diacetylenic Glutamate Lipids. **Langmuir** *7*, 584-589.

56. D. F. O'Brien, T. Kuo, U. Liman, and H. Lamparski. 1991 Polymerized Supramolecular Assemblies. **Polymer Journal** *23*, 619-628.

57. D. A. Frankel and D. F. O'Brien. 1991. Supramolecular Assemblies of Diacetylenic Aldonamides. **J. Am. Chem. Soc.***113*, 7436-7437.

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139. A.L. Grudinin, S.C. Arzberger, and D.F. O'Brien. 2001. Efficient Synthesis of Polymerizable Lipids for the Formation and Stabilization of Organic Zeolites. **Chem. Commun.**, submitted for publication.

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146. M. Romanowski, X. Zhu, K. Kim, V.J. Hruby, and D.F. O'Brien. 2002. Interaction of Enkephalin Peptides with Anionic Model Membranes. **Biochim. Biophys. Acta**, *1558*, 45-53.

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149. S.W. Jeong, D.F. O'Brien, G. Oröadd, and G. Lindblom 2002. Encapsulation and Diffusion of Water Soluble Dendrimers in a Bicontinuous Cubic Phase. **Langmuir** *18*, 1073-1076.

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152. V. Boguslavsky, M. Romanowski, A.W. Lipkowski, A. Misicka, V.J. Hruby, and D.F. O'Brien. 2001. Lipid Bilayer Permeability of Truncated Analogues of the Highly Potent Analgesic Peptide, Biphalin. **J. Med Chem.** submitted for publication.

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157. D. Yang and D.F. O'Brien 2002. Polymerized Bicontinuous Cubic Nanoparticles (Cubosomes) from a Reactive Monoacylglycerol. **J. Am. Chem. Soc.** *124*, submitted for publication.

INVITED PLENARY LECTURES

Gordon Conference on Micelles and Membranes, 1983. Reconstitution of Light Amplification Systems in Bilayer Membranes.

New York Academy of Sciences Conference on Polymers and Drug Delivery Systems, 1984. Preparation and Characterization of Polymerized Liposomes.

Gordon Conference on Chemistry of Interfaces, 1984. Polymerization of Vesicles.

Carl S. Marvel Symposium, University of Arizona, Tucson, 1985. Polymerized Vesicles: New Materials and Directions for Chemistry and Biology.

U. S.-Japan Polymer Symposium, Kyoto, Japan, 1985. Polymerized Vesicles: New Materials and Directions for Chemistry and Biology.

Gordon Conference on Synthetic Membranes, 1986. Polymerized Supramolecular Assemblies.

Moretonhampstead Conference on High Polymers, Great Britain, 1986. Polymerized Supramolecular Assemblies.

The Sixth Science and Technology Forum, Hakone, Japan, 1987. Membrane Reconstitution in Polymerized Membranes.

American Chemical Society Symposium on Polymerizations in Organized Media, New Orleans ACS Meeting, 1987. Permeability of Polymerized Vesicles.

German Chemical Society Symposium on Polymers and Biological Functionality, Bad Nauheim, FRG, 1988. Polymerized Model Biomembranes.

Gordon Conference on The Chemistry of Supramolecules and Assemblies, 1989. Chairman.

International Symposium on the Self-Assembly of Organic Compounds, Fukuoka, Japan, 1989. Polymerized Supramolecular Assemblies.

UCLA Symposia on Molecular and Cellular Biology on the Biochemistry and Molecular Biology of Biosensors and Bioprobes, Frisco, CO, 1990. Reconstitution of Proteins in Polymerized Bilayer Membranes.

Third SPSJ International Polymer Conference, Nagoya, Japan, 1990. Polymerized Supramolecular Assemblies.

Gordon Conference on The Chemistry of Supramolecules and Assemblies, Oxnard, CA,1991. Two-Dimensional Polymerizations.

American Chemical Society Symposium on Complex Fluids sponsored by the ACS Colloid Science Division, Atlanta, GA, 1991. Photoinduced Transformations of Lamellar to Nonlamellar Assemblies.

American Chemical Society Symposium on Advances in Biomimetic Chemistry sponsored by the ACS Polymer Chemistry Division, Atlanta, GA, 1991.

Gordon Conference on Reactive Polymers, Newport, RI, 1991. Two-Dimensional Polymerizations.

American Society of Photobiology Annual Meeting, Macro Island, FL, 1992. Photoinduced Reorganization of Bilayer Membranes.

American Chemical Society Symposium on New Macromolecular Architectures and Supramolecular Polymers, sponsored by the ACS Polymer Chemistry Division, Denver, CO, 1993.

American Chemical Society Award Symposium, sponsored by the ACS Polymer Chemistry Division, San Diego, CA, 1994.

Short Course on the Polymerization of Supramolecular Assemblies (5 Lectures), Max Planck Institut für Polymerforschung, Mainz, Germany, 1995.

American Chemical Society Award Symposium, sponsored by the ACS Polymer Chemistry Division, New Orleans, LA, 1996.

Inter-American Photochemical Society Biennial Meeting, Foz do Iguaçú, Brazil, 1996.

Seventh Annual Symposium for Photoinduced Charge Transfer: Reactive Processes in Organized Media, sponsored by the NSF Center for Photoinduced Charge Transfer, Rochester, NY. 1996.

Gordon Conference on Polymers, Ventura, CA, 1997. Novel Polymer Architectures via Supramolecular Assemblies.

Gordon Conference on The Chemistry of Supramolecules and Assemblies, Newport, RI., 1997.

American Chemical Society Colloid and Surface Science Symposium on Supramolecular Structure in Confined Geometries, Dallas, TX. 1998.

European Research Conference on Reactivity in Organized Microstructures, Weisbaden-Naurod, July, 1998.

Southern Illinois Materials Chemistry Conference, Carbondale, IL, October, 1998

American Chemical Society Symposium on Two-Dimensional Polymer Microstructures, sponsored by the ACS Analytical Division, Anaheim, CA, March, 1999.

American Chemical Society Symposium on Novel Surfactants sponsored by the ACS Colloid and Surface Division, New Orleans, August, 1999.

MITTI Sponsored Conference on New Polymers and their Nano-Organized Systems, Tokyo, October, 1999.

Materials Research Society Symposium on Materials Science of Phospholipid Assemblies, Boston, MA, November, 1999.

American Chemical Society Symposium on Molecularly Ordered Networks sponsored by the ACS Division of Polymeric Materials, San Francisco, CA, March, 2000.

International Conference on Porphyrins and Phthalocyanines, Symposium on Phthalocyanines as Materials, Dijon, France, June, 2000.

TEACHING

Semester		Course	
Fall	1987	Chemistry of Chain Polymerizations	Chem 642b
Spr	1988	Organic Reaction Mechanisms	Chem 541
Fall	1988	Chem. of Step and Ring Opening Polym.	Chem 642a
Spr	1989	Organic Reaction Mechanisms	Chem 541
Fall	1989	Chemistry of Chain Polymerizations	Chem 642b
Spr	1990	Organic Photochemistry	Chem 646
Fall	1990	Chem. of Step and Ring Opening Polym.	Chem 642a
Spr	1991	Honors Organic Chemistry	Chem 242b
Fall	1991	Chemistry of Chain Polymerizations	Chem 642b
Spr	1992	Honors Organic Chemistry	Chem 242b
Fall	1992	Chem. of Step and Ring Opening Polym.	Chem 642a
Spr	1993	Honors Organic Chemistry	Chem 242b
Fall	1993	Chemistry of Chain Polymerizations	Chem 642b
Spr	1994	Honors Organic Chemistry	Chem 242b
Fall	1994	Chem. of Step and Ring Opening Polym. Organic Chemistry Seminar	Chem 642a Chem 696c
Spr	1995	Honors Organic Chemistry	Chem 242b
Fall	1995	Sabbatical	
Spr	1996	Organic Reaction Mechanisms	Chem 541
Fall	1996	Chemistry of Chain Polymerizations	Chem 642b
Spr	1997	Organic Reaction Mechanisms	Chem 541
Fall	1997	Chem. of Step and Ring Opening Polym.	Chem 642a
Spr	1998	Organic Reaction Mechanisms	Chem 541

Fall	1998	Chemistry of Chain Polymerizations	Chem 542b
Spr	1999	Organic Reaction Mechanisms	Chem 541
Fall	1999	Chem. of Step and Ring Opening Polym.	Chem 542a
Spr	2000	Organic Reaction Mechanisms	Chem 541
Fall	2000	Chemistry of Chain Polymerizations	Chem 542b
Spr	2001	Majors Organic Chemistry	Chem 246b
Fall	2001	Chem. of Step and Ring Opening Polym.	Chem 542a
Spr	2002	Majors Organic Chemistry	Chem 246b

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- Biophysics Program Executive Committee - '91,'92
- Task Force on the Benefits of Graduate Education - '92
- Graduate Council - '96, '97, '98

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- Faculty Compensation Committee - '94
- Graduate Recruiting Committee - '87, '88, '89, '90, '91, '92, '96, '97 (Chair '88, '89, '92)
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- Dr. Polt P&T Committee - '93
- Dr. Mash P&T Committee (Chair) - '98
- Dr. McGrath Advisory Committee (Chair) - '98, '99
- Dr. McGrath P&T Committee - '00
- Dr. Zheng Advisory Committee - '98, '99, '00
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- Internal Departmental Review Committee - '88
- Staff Review Committee (Chair) - '88
- EPR Users Committee - '92
- Mass Spec Users Committee - '00, '01

Primary Reviewer

- Nature
- J. Am. Chem. Soc.
- Macromolecules
- Langmuir
- J. Org. Chem.
- Biochemistry
- Bioconjugate Chem.

Biochim. Biophys. Acta
Chem. Phys. Lipids
J. Polymer Science
J. Phys. Chem.

Proposal Review
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Editorial Advisory Board
Supramolecular Science '93-'98
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Advisory Board of the American Chemical Society-Petroleum Research Fund: 1992-1998.

Special Reviewer for Biophysical Chemistry Study Section, National Institutes of Health

Chairman 1989 Gordon Research Conference: "Chemistry of Supramolecular Assemblies"

Co-Chairman 1995 Eleventh Biennial Carl S. Marvel Symposium: "New Polymerizations for New Molecular Architectures"